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Analytical quality assessment and method comparison of immunoassays for serum cobalamin and folate measurement in dogs and cats

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Introduction

- The TOSOH assays (TOSOH Bioscience) are two competitive enzyme immunoassays for the measurement of serum folate (FOL) and vitamin B12 (B12), originally labelled for use in human medicine.
- The analytical quality of this assay needs to be evaluated prior to use in cats and dogs.

Objectives

- To assess the analytical performance of the TOSOH immunoassays for measurement of folate and vitamin B12 in cat and dog serum, by means of linearity, imprecision and recovery.
- To carry out a method comparison study on clinical samples, using the Immulite assays (Siemens Healthcare) as the reference method. These chemiluminescence immunoassays have been previously validated for use in cats and dogs.

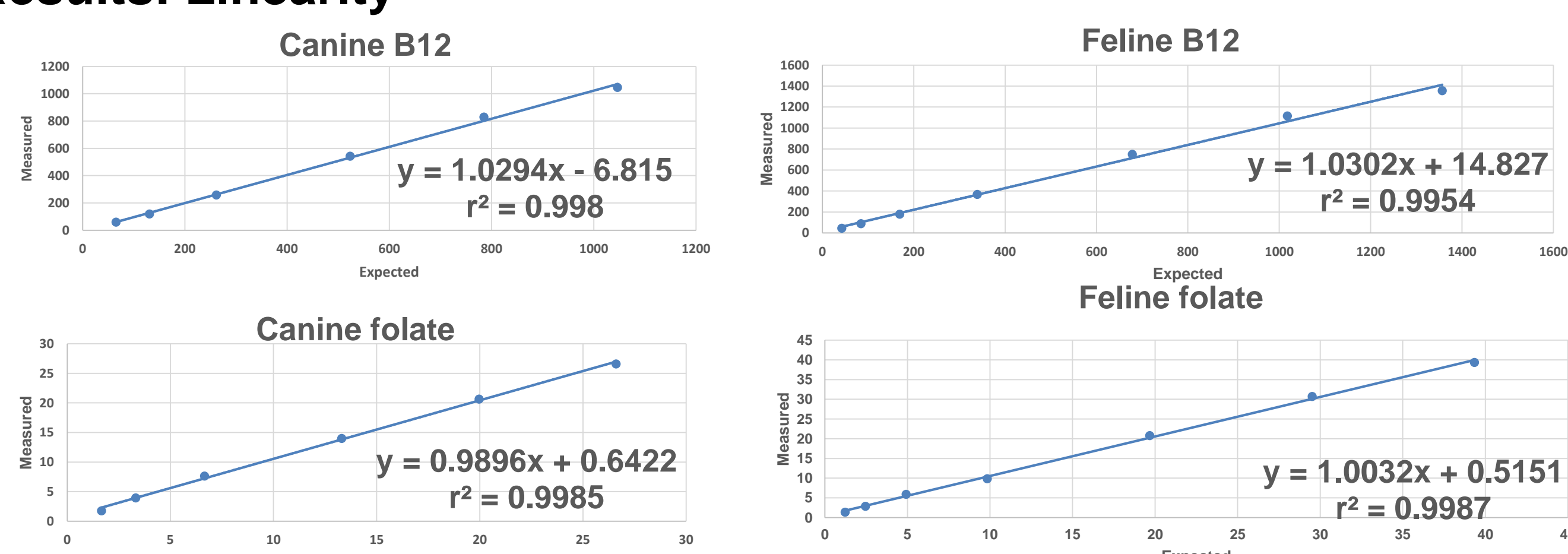
Materials and methods

- Samples used:**
 - Linearity, imprecision, recovery studies: high/medium/low B12/FOL canine and feline pools were created from stored frozen serum.
 - Imprecision study: high/medium/low B12/FOL quality control materials (QCM) were also used.
 - Method comparison: fresh canine and feline serum was used.
- Linearity:** High cat/dog B12/FOL pools were serially diluted with diluent buffer and FOL/B12 until the analyser could no longer detect the analyte. All data points were measured in duplicate.
- Imprecision:** Assessed using each level of QCM and each pool.
 - Within-run: samples analysed 10-13 times in a row, mean, SD and CV calculated. Between-run: samples analysed in duplicate, once daily for 10 to 15 days
 - For the QCM (between-run), bias and observed total error (TEobs) were also calculated.
- Recovery:** High, medium and low pools were mixed with one another in equal proportions, [B12] and [FOL] measured in duplicate and recovery percentage calculated.
- Method comparison:** Fresh serum samples from 39 dogs and 29 cats were tested on the reference (Immulite 2000) and TOSOH analysers (AIA-900) on the same day and results compared.

Statistics

- Linearity was assessed by plotting measured vs. expected results.
- Imprecision was evaluated using intra- and inter-assay coefficients of variation (CVs, %), bias and TE(obs).
- For the method comparison, data were tested for Gaussian distribution, then correlation (Spearman for B12/Pearson for FOL) and regression analysis (Deming and Passing Bablok regression) were carried out. This was followed by Bland-Altman analysis.

Results: Linearity



Results: Imprecision

	B12			Folate		
	QCM	Dog pools	Cat pools	QCM	Dog pools	Cat pools
Within-run CV	≤ 2.8%	≤ 4.1%	≤ 9.3%	≤ 9%	≤ 5.2%	≤ 2.9%
Between-run CV	≤ 4%	≤ 6%	≤ 6.8%	≤ 8%	≤ 5.4%	≤ 7.8%
TE(obs)	≤ 13%			≤ 31.4%		

→ TE(a) for B12: ≤ 30%^a so TE(obs) ≤ TE(a). CV<15%^{b,c}

→ TE(a) for Folate: ≤ 39%^a so TE(obs) ≤ TE(a). CV<15%^{b,c}

Results: Recovery

The average recoveries were:

→ B12: 99% (dog), 100% (cat) (ranges 97-101% and 95-106% respectively).

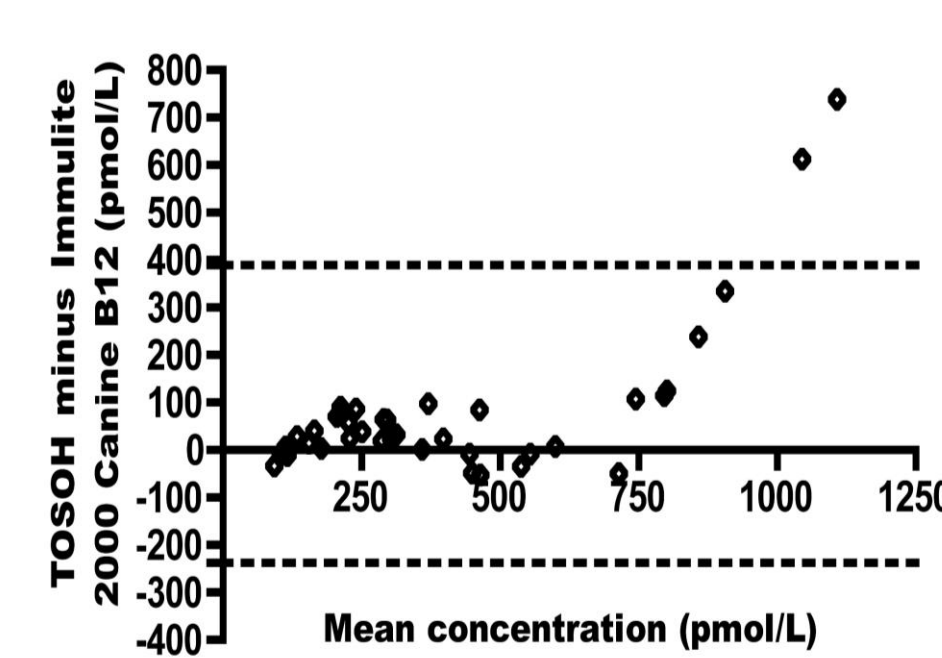
→ Folate: 101% (dog), 98% (cat) (ranges 100-102% and 96-102% respectively)

Results: Method comparison

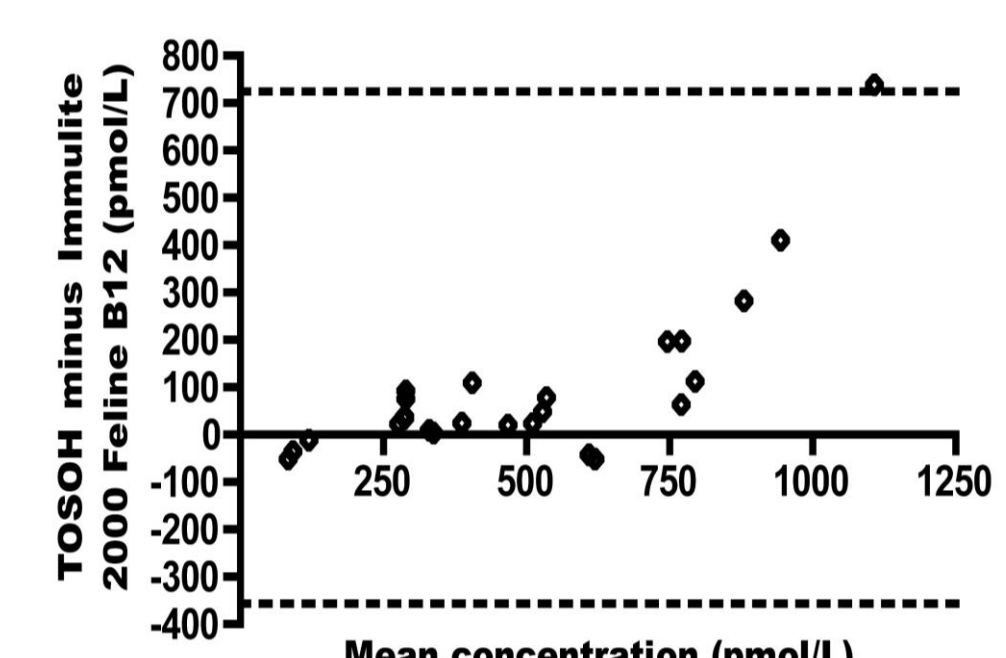
Ranges of sample concentrations and medians for the TOSOH and Immulite, and results of correlation and Deming/Passing Bablok regression:

Analyte	TOSOH median	TOSOH range	Immulite median	Immulite range	Correlation r value	Slope estimate	95% CI	y intercept estimate	95% CI
B12 canine	328	76 to >1476	291	<111 to >738	0.98 (p<0.001)	1.19	1.04 to 1.38	-17.57	-58.29 to 18.67
B12 feline	552	58 to >1476	499	<111 to >738	0.97 (p<0.001)	1.69	1.29 to 2.30	-160.98	-415.59 to 49.21
FOL canine	22.9	9 to >45.4	23.8	6.7 to >54.4	0.88 (p<0.0001)	0.75	0.60 to 0.88	4.96	1.19 to 8.74
FOL feline	30.1	9.4 to >45.4	34	9.8 to >54.4	0.92 (p<0.0001)	0.81	0.67 to 0.94	1.91	-2.91 to 6.73

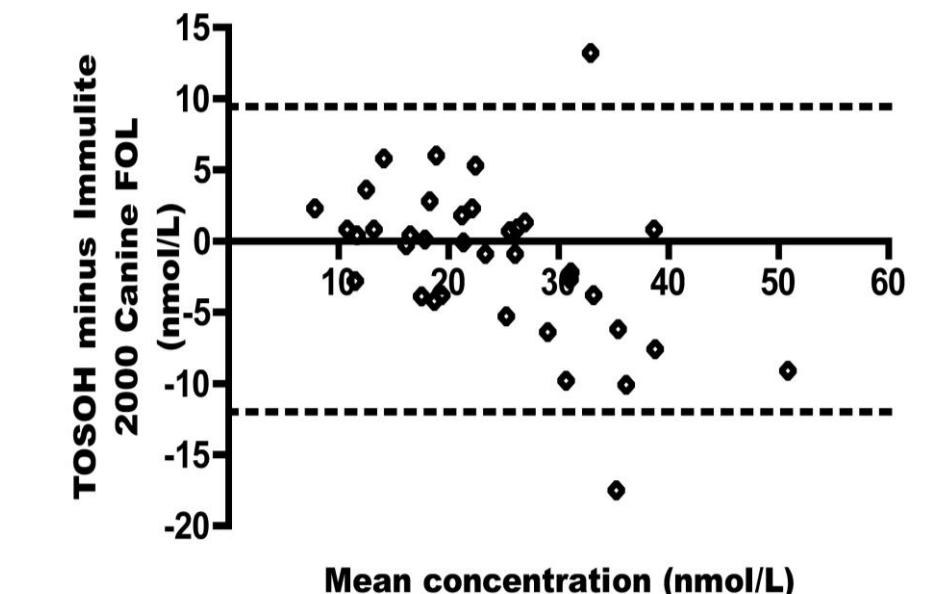
Bland Altman plot B12 canine. Bias = 75 pmol/L



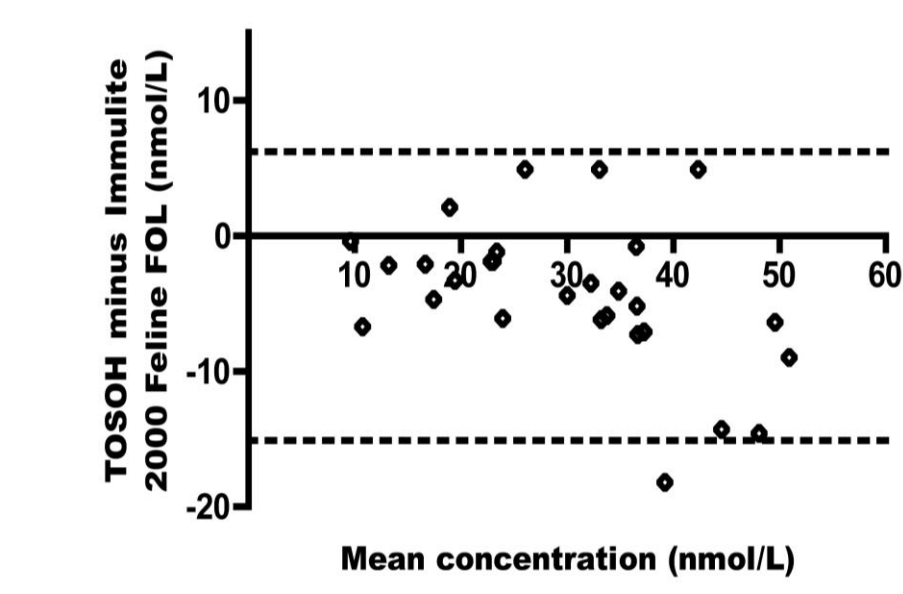
Bland Altman plot B12 feline. Bias = 184 pmol/L



Bland Altman plot Folate canine. Bias = -1 nmol/L



Bland-Altman plot Folate feline. Bias = -4 nmol/L



Conclusions

- The TOSOH immunoassays were linear over a wide range of concentrations, with high r^2 values.
- The assays met acceptability criteria for imprecision and bias. Recovery results were excellent.
- Analysis of 95% CI for intercept and slope estimates indicated the presence of proportional and constant error for B12, and proportional error for FOL.
- Despite correlations being excellent (B12) or good (FOL), the TOSOH assays cannot be used interchangeably with the reference method and require specific reference intervals.